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Signalling, inflammation and arthritis

MAPKs and their relevance to arthritis and inflammation

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Rheumatoid arthritis (RA) is a chronic autoimmune disease in which imbalances in pro- and anti-inflammatory cytokines promote the induction of autoimmunity, inflammation and joint destruction. The importance of inflammatory cytokines in the pathogenesis of RA has been underscored by the success of biologics that act to block the effects of cytokines, such as tumour necrosis factor-α, interleukin (IL)-1 or IL-6, in treating disease. Mitogen-activated protein kinases (MAPKs) have been implicated as playing key regulatory roles in the production of these pro-inflammatory cytokines and downstream signalling events leading to joint inflammation and destruction. This article reviews the evidence that MAPKs play important roles in the pathogenesis of RA and discusses their therapeutic potential as drug targets.

Key words: Mitogen-activated protein kinases, Extracellular signal-regulated kinases, C-jun N-terminal kinase, p38 Mitogen-activated protein kinase, Rheumatoid arthritis, Cytokines.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease in which imbalances in pro- and anti-inflammatory cytokines promote the induction of autoimmunity, inflammation and joint destruction [1, 2]. The initial stages of autoimmunity, which can precede articular inflammation by several years, are the result of breakdowns in B- and T-cell tolerance that can occur at a number of central or peripheral checkpoints and are evidenced by the production of rheumatoid factors [antibodies specific for immunoglobulin G (IgG)] or anti-citrullinated peptide antibodies. Poorly defined physiochemical, genetic and environmental factors then drive the development of articular disease that recruits a wide range of activated immune effector cells [including macrophages, mast cells, neutrophils, B-, T- and natural killer (NK) cells] into the affected joint(s). Cytokines [including the master regulators interleukin (IL)-1, IL-17 and tumour necrosis factor-α (TNFα)] and downstream inflammatory mediators [such as prostaglandins and the matrix metalloproteinases (MMPs)] produced by such cellular infiltrates promote the autoimmunity and chronic inflammation of the synovium that is required for the ultimate destruction of the cartilage and the underlying bone driven by synovial fibroblasts, chondrocytes and osteoclasts [1, 2]. Synovial inflammation and bone destruction are intimately linked as IL-1 and TNF-α drive production of MMPs by synovial fibroblasts to further promote inflammation and facilitate invasion of articular cartilage, whilst IL-1 and IL-17 convert chondrocytes to a phenotype that degrades, rather than synthesizes, matrix, resulting in cartilage destruction [1, 3, 4]. Similarly, TNF-α and IL-17 induce the production of M-CSF that is required for the early differentiation of osteoclasts, and along with IL-1, IL-6 and prostaglandin E2 (PGE2) up-regulates receptor activator of nuclear factor kappa B ligand (RANKL) expression on synovial fibroblasts to mediate differentiation of the bone-resorption capabilities of osteoclasts. Moreover, the potential for compensatory new bone formation is essentially ablated in RA as TNF-α also acts to suppress the maturation and function of bone-producing osteoblasts [1, 5–8].

The importance of inflammatory cytokines in the pathogenesis of RA has been underscored by the success of biologics in treating disease by blocking the effects of cytokines such as TNF-α, IL-1 or IL-6. However, there remains a significant unmet clinical need for new therapies as existing regimens are effective in only a proportion of patients and crucially leave the host at significantly higher risk of overwhelming infection, e.g. tuberculosis, entailing careful screening and limitations of use in some populations. Moreover, the high cost, short half-life and i.v. application of these drugs have made it necessary to consider alternative treatments such as orally active small molecule inhibitors of signalling cascades relating to these important inflammatory mediators. Much interest has focused on inhibitors of the mitogen-activated protein kinases (MAPKs) primarily because they have been implicated as key regulators of pro-inflammatory cytokine (e.g. IL-1, IL-6, IL-12, IL-23 and TNF) production as well as playing crucial roles in signalling via the B cell antigen receptor (BCR), TCR/CD28 and toll-like receptor (TLR), IL-1, IL-17 and TNF-α receptors [9–14]. Indeed, p38 MAPK was initially discovered as the target of pyridinyl imidazoles [15, 16], a class of compounds that inhibit lipopolysaccharide (LPS)-stimulated production of TNF-α and IL-1 by human monocytes and p38 MAPK inhibitors are currently undergoing phase II trials for RA (www.sciosinc.com/scios/pr_1046376591; www.vpharm.com/Pressreleases2006/pr030806.html). This article reviews the evidence that MAPKs play important roles in the pathogenesis of RA and discusses their therapeutic potential as drug targets.

MAPK

MAPKs comprise a family of highly conserved serine/threonine protein kinases that have been implicated in the regulation of key cellular processes including gene induction, cell survival/apoptosis, proliferation and differentiation as well as cellular stress and inflammatory responses. There are three major classes of MAPKs in mammals, the extracellular signal-regulated kinases (ERKs) and the two stress-activated protein kinase (SAPKs) families, c-jun N-terminal kinase (JNK) and p38. MAPKs are activated via a signalling cascade that is conserved from yeast to mammals [17, 18]. Thus, stimulation of MAPKs requires the upstream activation of a MAPK kinase (termed MAPKK, MEK or MKK) and an MAPK kinase kinase (termed MAPKKK, MEKK or MKKK). MKKKs are serine/threonine protein kinases that phosphorylate and activate MKKs, whilst MKKs are dual-specificity protein kinases that phosphorylate the threonine and tyrosine residues of a conserved T–X–Y motif of the activation loop of MAPKs (Fig. 1). The X residue is different in each class of MAPKs: ERK has a threonine–glutamic acid–tyrosine (T–E–Y) motif, JNK has a threonine–proline–tyrosine (T–P–Y) motif

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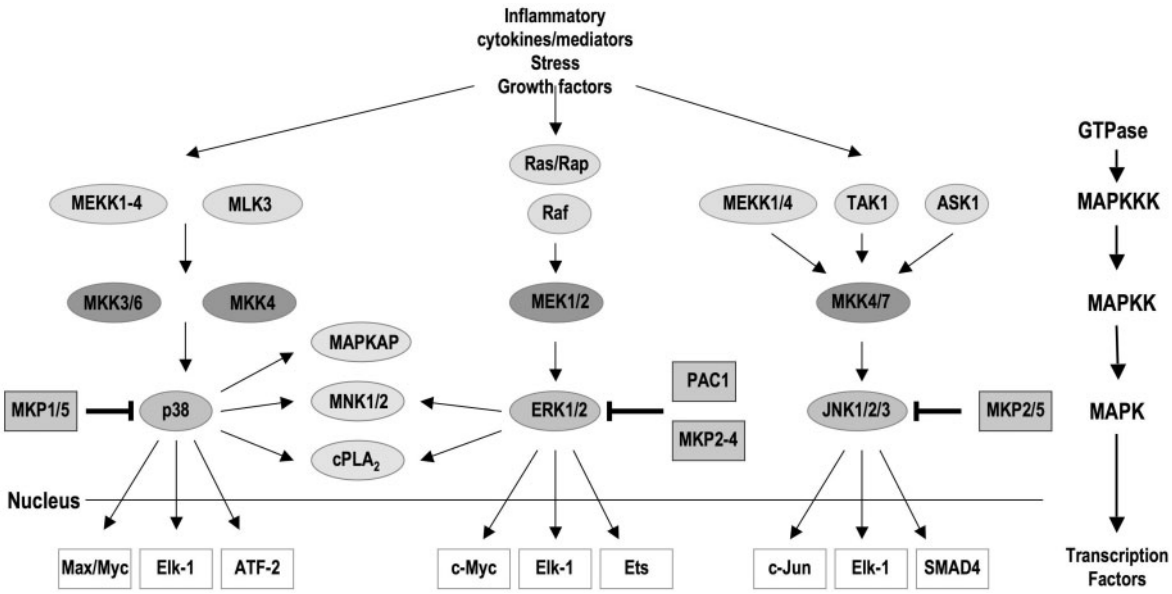

FIG. 1. The ERK, JNK and p38 MAPK cascades. There are three major classes of MAPKs in mammals, the ERKs and the two SAPKs families: JNK and p38. MAPKs are activated via a signalling cascade that is conserved from yeast to mammals in which stimulation of MAPKs requires the upstream activation of a MAPK kinase (termed MAPKK, MEK or MKK) and a MAPK kinase kinase (termed MAPKKK, MEKK or MKKK). Activation of the MAPKs results in phosphorylation of a specific repertoire of cytoplasmic and nuclear target proteins including various transcription factors. Negative feedback mechanisms including MAPK phosphatases (MKPs) exist to ensure MAPK enzymes are not activated constitutively. ASK, apoptosis signal-regulating kinase; ATF, activating transcription factor; ERK, extra-cellular signal-regulated kinase MAPK; JNK, c-Jun NH_2-terminal kinase MAPK; MEK, MAPK kinase; MEKK, MEK kinase; MLK, mixed lineage kinase; MNK, MAPK interacting serine/threonine kinase; MAPKAP, MAPK activated protein kinase; $cPLA_2$, cytosolic phospholipase A_2; MKK, c-Jun NH_2-terminal kinase MAPK kinase; Elk, Ets-like transcription factor; Ets, E26-AMV virus oncogene cellular homologue; TAK, TGFβ-activated kinase; PAC, phosphatase of activated cells; MKP, MAPK phosphatase.

and p38 has a threonine–glycine–tyrosine (T–G–Y) motif. Phosphorylation of the T–X–Y motif activates the MAPKs, allowing phosphorylation of a specific repertoire of cytoplasmic and nuclear proteins including various transcription factors. The substrate specificity of MAPKs is influenced by the X residue of the T–X–Y motif and by the amino acids surrounding this motif since this region can affect the availability or conformation of the substrate-binding groove [17, 18]. Each MAPK enzyme can be activated by a number of different upstream MKKs and MKKKs, each with unique properties regarding its regulation, substrate specificity and kinetics of activation providing specificity and allowing activation by a broad range of immunological stimuli acting via the antigen and co-stimulatory receptors, FcRs, TLRs and cytokine receptors [10, 19–23].

Negative feedback mechanisms exist to ensure that MAPK enzymes are not activated constitutively. For example, MAPKs can induce at least three different types of protein phosphatases that dephosphorylate and inhibit MAPK: dual-specificity phosphatases (DUSPs), threonine phosphatases and tyrosine phosphatases [24–27]. For example, the MAPK phosphatases for ERK2 include the dual-specificity phosphatase MKP3, the threonine phosphatase PP2A and the tyrosine phosphatase HePTP [28]. The DUSPs form a family of proteins that can dephosphorylate both the threonine and tyrosine residues of the T–X–Y motif [27]. The activity of DUSPs is largely regulated at the transcriptional level and the expression of specific protein phosphatases allows the selective inhibition of particular MAPK proteins since each phosphatase has a precise substrate specificity [29]. For example, procaspase-activating compound 1 (PAC-1) dephosphorylates ERK and p38, whilst MKP-1 acts on all three families of MAPKs. In contrast, MKP-2 preferentially dephosphorylates ERK and JNK and MKP-M dephosphorylates JNK. Indeed, each member of the DUSP family has a unique set of properties including tissue distribution, subcellular localization, regulation and substrate specificity [27, 29]. For example, PAC-1 is a DUSP predominantly located in the nucleus of haematopoietic cells that preferentially dephosphorylates ERK MAPK, acting on the tyrosine residue of the T–E–Y motif prior to dephosphorylating the threonine residue. The expression of PAC-1 in B- and T-cells is increased by various mitogenic stimuli since the transcription of the *pac-1* gene can be activated by transcription factors that are regulated by ERK MAPK. DUSPs often have a short half-life to prevent excessive inhibition of MAPK. However, the degradation of DUSPs can also be regulated by MAPKs thus creating the potential for prolonged activation of ERK [24–27, 29].

Whilst all three classes of MAPKs have been shown to be expressed in the synovial tissue of RA and osteoarthritis patients, phosphorylation and therefore activation of the enzymes could be detected only in tissue from RA patients, implicating a role(s) for these MAPKs in the pathogenesis of this disease [30]. Interestingly, the activated form of the three classes of MAPKs displayed distinct localization patterns in synovial tissue from RA patients. Thus, phospho-p38 MAPK was expressed in both the endothelium of the synovial microvessels and in the cells of the synovial lining layer. In contrast, activation of ERK MAPK was detected in mononuclear infiltrates and cells within the synovial lining area around synovial microvessels, whilst JNK MAPK was shown to be active predominantly in mononuclear cell infiltrates in the sublining areas [30]. TNF-α, IL-1 and IL-6 have been proposed to play important roles in promoting RA by inducing production of IL-8, MMPs and adhesion molecules by many of the cells present in the synovium, thereby further enhancing cell infiltration, inflammation and cartilage destruction. To address the role(s) of MAPKs in these processes, the ability of such pro-inflammatory cytokines to induce MAPK activation in synovial fibroblasts and chondrocytes was tested *in vitro*. TNF-α, IL-1 and IL-6 were found to activate all three classes of MAPKs in synovial fibroblasts [30] and TNF-α and IL-1 were similarly found to activate ERK, JNK and p38 MAPK [31–33], with consequent induction of MMP-1 and MMP-13 in chondrocytes [33–35]. Moreover, TNF-α and IL-1 were shown to play important *in vivo* roles in the activation of p38 and ERK MAPKs in inflamed

synovial tissue as TNF-α or IL-1 blockade reduced the activation of these MAPKs in the synovium of TNF-α-expressing transgenic mice [36]. Furthermore, MAPK phosphatase-1 (MKP-1) deficiency exacerbates murine collagen-induced arthritis (CIA), underlining the importance of negative feedback mechanisms in keeping MAPK signalling and consequent inflammation in check [37]. However, PAC-1 (DUSP2) knockout mice exhibit reduced inflammatory responses and less-severe histological changes in the joints. These rather paradoxical results in PAC-1-deficient mice appear to reflect unforeseen reductions in ERK and p38 MAPK activity due to increased JNK MAPK signalling [25], highlighting the unpredictable cross-talk that can occur amongst MAPK cascades in the presence of inhibitors [14, 19] and that dictates that side effects are likely to arise even with highly isoform-specific inhibitors. Nevertheless, collectively, these findings indicate that in inflamed synovial tissue, all three MAPKs are (aberrantly) activated and the differences in their activation patterns suggest they may each play specific roles in the disease process.

ERK MAPK

Specific inhibitors (PD98059 and PD198306: Pfizer Inc., CT, USA and U0126: DuPont Inc., DE, USA) that target ERK MAPK by inhibiting the activation of the upstream regulators, MEK1/2 [38, 39] have been available for over 10 yrs but there is only limited information available concerning their therapeutic potential in chronic inflammatory conditions such as RA. For example, there are no reports that these compounds reduce inflammation or joint destruction in animal models of arthritis although PD098059-reduced nociceptive responses in an adjuvant-induced monoarthritis in rats, suggesting that ERK plays an important role in transducing chronic inflammatory articular pain [40]. However, ERK MAPK inhibitors have been found to be successful in reducing inflammation in an ear oedema model in mice and in an experimental osteoarthritis model in rabbits [41, 42]. Nevertheless, ERK MAPK has been shown to be activated in synovial fibroblasts following stimulation with IL-1, TNF-α and fibroblast growth factor (FGF) and is indeed found to be activated in mononuclear cell infiltrates and synovial fibroblasts in synovial tissue from RA patients [30]. As ERK is known to be involved in the regulation of IL-6, IL-12, IL-23 and TNF-α synthesis [13, 14], these *in vitro* findings suggest a possible involvement of ERK in joint damage associated with pro-inflammatory cytokine production by macrophages. Moreover, ERK MAPK signalling could also play a role in the maintenance phase of disease, by promoting pannus formation, due to its role in phospholipase A_2 induction, prostaglandin synthesis and resultant chemotaxis of cells into the joint [30].

Indeed, paw extracts from transgenic mice over-expressing TNF-α exhibited higher levels of phosphorylated, activated ERK MAPK relative to those obtained from wild-type mice and such ERK MAPK activity was found predominantly in macrophages and fibroblasts within the synovium [36]. At present, the potential role of ERK MAPK in such joint inflammation and destruction is not clear. However, IL-1 induced MMP-1 gene expression by rabbit synovial fibroblasts requires both NF-κB and ERK MAPK activation [43, 44]. Similarly, IL-1-induced AP-1 activation and subsequent MMP-1 induction in synovial fibroblasts from RA patients was slightly decreased by inhibition of ERK MAPK, presumably due to suppression of its downstream effector, c-Fos [45]. Moreover, the MEK1/2 inhibitor PD198306 decreased MMP-1 production by chondrocytes in a rabbit model of osteoarthritis [42] and the ERK inhibitors, U0126, and to a lesser extent PD98059, ablated TNF-α-induced MMP-13 (mRNA and protein) production by chondrocytes from patients with osteoarthritis [33]. Interestingly, studies with human osteoblasts indicated that culture with the ERK inhibitor PD98059 resulted in stimulation of MMP-13 production from these cells [46]. Thus, the induction of MMPs, which remove matrix components such as cartilage in order for osteoclasts to attach to bone resulting in bone resorption [46], may be a target for therapeutic intervention by ERK inhibitors.

JNK MAPK

Specific JNK MAPK inhibitors have only recently been developed and so there is also relatively little evidence regarding the therapeutic potential of JNK MAPK in RA. However, recent work employing a specific inhibitor (SP600125) and cells from JNK1 or JNK2 knockout mice has suggested that JNK MAPK also appears to play a major role in the regulation of collagenase production by synovial fibroblasts [45, 47]. Thus, for example, SP600125 suppressed IL-1-induced and JNK MAPK-mediated c-Jun phosphorylation and AP-1 up-regulation, resulting in consequent down-regulation of MMP-1 expression [45]. Similarly, synovial fibroblasts from JNK1 or JNK2-deficient mice were found to express reduced levels of MMP-3 and MMP-13. In addition, JNK1 appears to play a role in driving osteoclast differentiation as JNK1-, but not JNK2-, deficient osteoclast progenitors were unable to mature into bone-resorbing osteoclasts following exposure to RANKL stimulation [48]. Consistent with these findings, whilst prophylactic treatment with SP600125 in an adjuvant-induced arthritis model only modestly improved inflammation, it significantly reduced joint destruction [45]. The relative contributions of JNK1 and JNK2 to bone destruction *in vivo* are not clear, however, as mice defective in either JNK1 or JNK2 exhibited only marginally reduced joint inflammation and destruction [49, 50], suggesting that both isoforms may have to be targeted for effective therapy.

To identify potentially more inflammation-specific upstream targets in the JNK MAPK pathways, synovial fibroblasts were examined for the expression and activation of MAPKKs and MAPKKKs that regulate JNK MAPK signalling. This revealed that MKKK2 was the major kinase activated by IL-1 and the most potent inducer of the JNK MAPK cascade [51]. Moreover, although both MKK-4 and -7 were found to be activated downstream of MKKK2 by IL-1 [52], only MKK-7 was essential for IL-1-stimulated JNK MAPK activation and consequent c-Jun phosphorylation and AP-1 activation [53], suggesting that targeting of MKK-7 could allow specific suppression of harmful inflammation whilst leaving MKK-4-mediated activation of stress-related pathways intact.

p38 MAPK

p38 MAPK is generally considered to be the most promising MAPK therapeutic target for RA as p38 MAPK isoforms have been implicated in the regulation of many of the processes, such as migration and accumulation of leucocytes, production of cytokines and pro-inflammatory mediators and angiogenesis, that promote disease pathogenesis (reviewed in references [30, 54]). For example, inhibitors of p38 have been shown to block TNF-α, IL-1, IL-8 and cyclo-oxygenase-2 (COX-2) production by monocytes [16, 55], with p38 MAPK playing crucial roles in the regulation of IL-1 and TNF-α synthesis at both the transcriptional and translational levels [16, 56, 57]. Similarly, p38 MAPK regulates IL-1-stimulated production of IL-6 and IL-8 and induction of COX-2 and MMP-1 and -3 in fibroblasts [58–60] and IL-1-stimulated nitric oxide production by chondrocytes [61]. Moreover, p38 MAPK also appears to play a key role in regulating osteoclast differentiation induced by M-CSF/RANKL or TNF-α by down-regulating tartarate-resistant acid phosphatase (TRAP) mRNA levels [62, 63], although it does not appear to be required for the survival or function of mature osteoclasts [63]. In addition, inhibition of p38 MAPK activity suppresses the TNF-α/IL-1-mediated induction of IL-6 by osteoblasts [64] and chondrocytes [65] that plays a role in osteoclast formation and bone resorption [64, 66]. Finally, and consistent with reports of the crucial role of p38 MAPK in IL-17

TABLE 1. MAPK inhibitors in clinical trial

Target	Compound	Company	Disease	Clinical Trial
p38	BIRB 796	Boehringer Ingelheim GmbH	RA, Psoriasis, Crohn's disease	III (RA)[79, 96] http://www.invitrogen.com/content.cfm?pageid=10561
p38	RWJ67657	Johnson & Johnson		[80, 81]
p38	SCIO-496	Scios Inc.	RA, multiple myeloma	III (RA)[97] http://www.arthritissupport.com/library/showarticle.cfm/ID/644/t/Arthritis http://www.invitrogen.com/content.cfm?pageid=10561
p38	VX-745	Vertex Pharmaceuticals	RA	II [98] http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/01-04-2001/0001396593&EDATE
p38	VX-702	Vertex Pharmaceuticals	Acute coronary syndrome (ACS), RA	II (ACS)[99] http://www.vpharm.com/Pressreleases2004/pr101804.html http://www.invitrogen.com/content.cfm?pageid=10561
p38	SB681323	GlaxoSmith Kline	COPD, RA	I/II http://www.druglib.com/trial/33/NCT00380133.html
JNK + p38	CNI-1493	Cytokine Pharma Sciences Inc.	Crohn's disease	I [100]
JNK	CEP-1347	Cephalon	Parkinson's disease	I (neuro-degeneration) II/III (Parkinsons, discontinued) [101] http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/05-11-2005/0003595559&EDATE http://www.invitrogen.com/content.cfm?pageid=10561
MEK	AZD6244	AstraZeneca	Cancer	I/II http://www.medicalnewstoday.com/articles/44838.php http://www.invitrogen.com/content.cfm?pageid=10561
MEK	CI-1040	Pfizer Ltd	Cancer	I/II [102–104]

signalling [11, 12], IL-17-mediated enhancement of IL-6 production by osteoblasts following TNF-α stimulation is abrogated by inhibition of p38 MAPK [67].

The importance of these p38 MAPK targets *in vivo* has been demonstrated in a range of different animal models of RA. Thus, p38 MAPK inhibitors have been proven to be effective in prophylactically reducing clinical severity, paw swelling, inflammation, cartilage breakdown and bone erosion in the rat streptococcal cell wall arthritis model [68, 69], the CIA model in mice [70] and the adjuvant and CIA models in rats [71, 72]. Importantly, these disease-suppressing effects could also be seen in animals treated therapeutically [68–72], and as the inhibitors were able to inhibit further bone erosion in such regimens [70], these studies suggest that p38 MAPK inhibitors indeed have therapeutic potential for patients with well-established disease. The ability to inhibit further bone erosion is likely to be mainly due to the inhibition of osteoclast differentiation [63] as osteoclast numbers were shown to be reduced in the affected joints in animals treated with p38 MAPK inhibitors [70, 72, 73]. Moreover, the suppression of TNF-α and IL-1 production by inhibition of p38 MAPK may also contribute to reduced osteoclast numbers as both of these cytokines are known to induce osteoclast differentiation independently of RANKL [74–76]. Additional validation of p38 MAPK as a target as well as the identification of potential novel targets was provided by mice deficient in a downstream effector of p38 MAPK, MAPKAP kinase-2 (MK-2) as such mice showed lower severity scores and incidence in a CIA model [77]. These effects appeared to be due to MK-2 playing a crucial role in the induction of TNF-α as deletion of the MK-2 gene in mice led to a 90% reduction of serum TNF-α levels after LPS challenge [78].

Such validation of the therapeutic potential of p38 MAPK inhibitors in inflammatory disease has led to the development of a large number of inhibitory compounds, such as RWJ67657 and BIRB-796 have been tested in clinical studies in humans (Table 1) and found to be safe [79–83]. Indeed, a single dose application has proved sufficient at reducing p38 MAPK activation in leucocytes, cytokine production (IL-6, IL-8 and TNF-α), C-reactive protein release in serum as well as clinical symptoms (fever) upon endotoxin challenge in healthy volunteers [82, 83]. Hence, studies to evaluate the safety and efficacy of these and other compounds such as VX-745, VX-702 and SCIO-469 in patients with RA are currently underway with several companies [84]. To date, efficacy of these compounds in RA appears limited with respect to placebo controls and there are significant adverse reactions and hepatotoxicity problems (reviewed in reference [21]). Moreover, because p38 MAPK plays key roles in cellular processes unrelated to inflammation, it is likely that such compounds could result in serious side-effects. Thus, the search for more inflammation-specific p38 MAPK inhibitors is underway, focusing on the therapeutic potential of different isoforms of p38 MAPK (p38α and p38γ appear to be the dominant isoforms expressed and activated in synovial tissue from RA patients) and regulatory elements upstream of p38 activation [54, 85–87]. Thus, for example, both MKK-3 and MKK-6 have been found to be activated in synovial fibroblasts and the synovium of RA patients [88] and the use of dominant negative mutants revealed that they are both able to activate p38 MAPK in synovial fibroblasts [89]. The relative importance of MKK-3 *vs* MKK-6 in p38 MAPK signalling in inflammation, however, was dissected by exploiting MKK-3 knockout mice to show that LPS-stimulation of IL-1 and IL-6 production is not affected by MKK-3 deficiency *in vivo*. In contrast, MKK-3 deficiency not only leads to ablation of IL-1 and IL-6 production in response to TNF-α but also to reduced severity of disease in a passive model (K/BxN) of arthritis [90]. Taken together, these *in vivo* data suggest that MKK-3 is a more selective candidate target for inhibition of the p38 MAPK signalling pathway in inflammation.

Conclusions and future perspectives

Clearly, MAPKs play important roles in transducing inflammation and joint destruction and therefore are key molecular targets for therapeutic intervention in chronic inflammatory diseases such as RA. However, there are multiple isoforms of these kinases (ERK-1–8, JNK1–3 and p38-α, -β, -γ and -δ) that have been implicated in the regulation of a plethora of essential cellular responses [91–94], dictating that inhibitors that simply ablate ERK, JNK or p38 MAPK activities are likely to have serious side effects. Moreover, most of the current inhibitor compounds under trial are competitors for the adenosine triphosphate (ATP)-binding site of p38 MAPK and hence are likely to induce side effects due to cross inhibition of other classes of kinases *in vivo* [19]. Interestingly, the ERK/MEK inhibitors are not ATP competitive but rather act to prevent the MAPKKK, Raf1 from activating MEK and thus their observed relative lack of side effects probably reflects that they do not non-specifically inhibit other kinases *in vivo* [19]. Similarly, peptide-based approaches to disrupting JNK signalling by targeting scaffold protein interactions show promise but as yet have not been tested in *in vivo* models of inflammation [21]. The future therefore lies in the development of inhibitors that target inflammation-restricted MAPK signalling and, in particular, the aberrant inflammation

related to chronic disease whilst leaving intact the 'healthy' inflammation that is essential for fighting infection. The recent explosion in identifying isoforms of the major components (MAPKs, MAPKKs, MAPKKKs and DUSPs) of MAPK cascades [21, 24, 93–95] and the delineation of their specific roles in coupling individual receptors to particular ERK, JNK and p38 MAPK signals and their functional outcomes will ultimately lead to the unravelling of disease pathogenesis and the development of specific inhibitors that will provide novel, safe small-molecule therapeutics for arthritis.

Rheumatology key messages

- MAPKs play key roles in the molecular and cellular events underpinning the pathogenesis of RA.
- MAPKs are rational targets of drug design for novel therapies in RA.

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